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ON THE SURFACE TENSION CHANGES WHICH UNDERLIE MUSCULAR AND AMOEBOID MOVEMENT.

by

O. W. TIEGS

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(Submitted for publication 22nd January, 1926.)

The theory that alterations of surface tension underlie muscular and apparently protoplasmic movement in general has been developed by D'Arsonval, by Quincke, and notably by Julius Bernstein (3). The work of D'Arsonval is of especially great interest, in that it involved the application of the same principle as that which underlies the action of the capillary electrometer, i.e., a surface tension change accompanying an alteration in the quantity of electricity condensed as a double layer at the interface of two fluids.

The first attempt to fit a surface tension scheme into precise histological observations was made by T. B. Robertson (6). This theory, which is based on the description of a contracting sarcomere as given by Schäfer, depends on the same principle as that suggested by D'Arsonval, namely, a change of surface tension accompanying an alteration of charge on a condenser, as the excitation wave travels through the fibre; the condenser being constituted by the electrical double layer which must exist at the surface of the hyaloplasm, if this hyaloplasm contains ion-proteid compounds. I have proposed a mechanism, depending upon the same principle, but based on microscopic observations which led to conclusions somewhat different from those of Schäfer (8, 9). The conception involved in this theory is, that there occurs in muscle a change of surface tension accompanying an alteration of the quantity of electricty condensed at its surface, under the influence of lactic acid, liberated not directly on the surface, but in its neighbourhood. A theory, not differing essentially from this has also recently been suggested by A. V. Hill (5).[1] In the present paper I wish to discuss this surface tension scheme in greater detail.

[1] Hill has shown that the quantity of lactic acid formed by a muscle on excitation is quite inadequate to cover, even in a layer one molecule thick, the free surface necessary to develop a tension of the order observed in a muscle twitch. But in denying that surface tension can therefore play any part in the process of contraction, the fact seems to have been overlooked that a surface tension change can be produced by means other than the spreading of a substance

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The facts upon which the theory is based are the following:

1. The unit of contraction of the muscle fibre is the sarcomere, and not an invisible ultramicroscopic colloidal complex. This is proved by the fact that the sarcomere can be observed to undergo contraction as a result of a change in position of part of its fluid contents.

2. The middle third of a sarcomere can be shown to contain a substance which does not undergo change in position as the sarcomere passes from the relaxed into the contracted condition. This substance is recognizable by the fact that after fixation it is still doubly refracting to polarized light.

3. The sarcomere also contains a fluid (hyaloplasm), recognizable because of its affinity for haematoxylin. In the *relaxed* muscle this fluid occupies the region of the sarcomere (middle third), which is doubly refracting; it is also found in very small quantity at the ends of the sarcomere, immediately adjacent to Krause's membrane (see figure 3). For convenience the hyaloplasm of the middle of the sarcomere and at the ends will be referred to respectively as the movable and the residual hyaloplasm. Under very high magnification delicate streaks of a similarly stainable material are seen, connecting the movable with the residual hyaloplasm at either end of the sarcomere. These streaks are especially well seen in flying muscles of insects, where Schäfer first observed them. I have also seen them in leg muscles of insects (see figure 3).

When now the sarcomere is examined in the contracted state the movable hyaloplasm is seen to have retreated entirely from the middle of the sarcomere (which still remains doubly refracting, however), and to have accumulated at the ends of the sarcomere, in the region previously occupied by the residual

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over the active surface; for example, in the capillary electrometer, the surface electrification which produces the surface tension change is brought about by the application of a potential difference, at points distant from the active surface; and it seems to be precisely on this principle that the surface tension machine of muscle acts.

A theory of muscular contraction has recently been proposed by W. E. Garner (Proc. Roy. Soc. (B), 99 (1925), p. 40), in which it is maintained that muscular contraction is the result of a chemical interaction between the lactic acid and the substance of the anisotropic segments of the myofibrils, the liquid crystal structure of the latter being converted at its surface into a solid film, with the production of a tension parallel to the axis of the fibril. However successful this theory may be in explaining the process of shortening of a muscle, it encounters the fatal objection that histological observation definitely contradicts it. If numerous microscopic preparations of muscle fibres (especially insect muscle fibres) are made, sooner or later one will encounter the good fortune to obtain a fibre in which a contractile wave has been fixed. If the sarcomeres of successive striations along such a contractile wave be examined, the whole process of contraction can be followed without difficulty, and in such preparations it can be observed with unmistakable clearness that the process of contraction is the result of the movement of a fluid *within* the sarcomere. The theory as developed by Garner leads to the conclusion that an individual sarcomere, homogeneous to miscroscopic appearance, merely undergoes a shortening, while retaining its homogeneous appearance; miscroscopic observation shows beyond doubt that such is not the case.

A second difficulty that Garner's theory will encounter is that the sarcomeres should tend to swell in their mid-region (anistropic portion) when they contract, i.e., a cylindrical sarcomere will become cask-shaped. Microscopically it is found that precisely the opposite occurs (see, for example, figures 1 and 2).

hyaloplasm alone (compare figures 1 and 2; also 3 and 4). When successive sarcomeres in a fixed contractile wave are examined, all intermediate stages between complete relaxation and contraction can be observed.

It might be urged that the change in position of the movable hyaloplasm is only an apparent one, and that an alteration in the acidity of the middle and ends of the sarcomere underlies the reversal of the staining reaction, but this is disproved by the fact that the tissue, during its preparation for microscopic examination, undergoes treatment with acid reagents, etc., so that any such variation of acidity of its parts will be abolished long before the tissue is stained.

4. The following observation is of especially great importance: If successive sarcomeres along a fixed contractile wave are examined a very considerable movement of the hyaloplasm can be seen to have occurred, without the sarcomere having undergone any visible contraction. From this we must conclude that the movement of the hyaloplasm is not caused by contraction of the sarcomere, but that contraction of the sarcomere occurs only after a sufficiently extensive movement of hyaloplasm has occurred.

5. Bernstein (3) gives reasons for concluding that the surface tension change cannot occur on the surface of the myofibrillae, but that a larger, free surface, situated within the fibrillae, is necessary. Where is this free surface situated? Is it in the doubly refracting middle of the sarcomere, or is it to be sought for at the ends, adjacent to Krause's membrane? The histological observations are decidedly in favour of the view that it occurs in the middle of the sarcomere. Firstly, there is a substance present in this situation in large enough quantity to be recognizable by its double refraction in polarized light; and this doubly refracting substance cannot be the hyaloplasm, since its optical properties remain unchanged, even after the hyaloplasm has retreated from it. Secondly, the volume of the hyaloplasm in the contractile sarcomere appears to be considerably smaller than in the relaxed sarcomere (compare figures 3 and 4); in the contracted sarcomere it is also considerably more strongly staining than in the relaxed sarcomere. These observations find a ready interpretation if we assume that the doubly refracting material, which is relatively weakly staining, is porous, and presents a large free surface, into which the hyaloplasm extends.

We therefore regard the middle of the sarcomere as consisting of a highly porous substance; its cavity is connected by means of very fine capillary channels (observable under the microscope as the blue-staining longitudinal streaks above referred to), with a space at either end of the sarcomere, which presents relatively little free surface, and is probably to some extent collapsible; it might be in the form of a series of collapsible sacs, or of a loose spongework. During contraction the hyaloplasm is drawn from the doubly refracting porous mid-region of the sarcomere, through the fine longitudinal channels, into the collapsible spongework at the ends of the sarcomere. This spongework is dilated, and the sarcomere

thickens at its ends, and therefore shortens. Relaxation occurs when the hyaloplasm moves back into the central porous substance.

If we could locate the large free surface at the ends of the sarcomere, rather than in its middle, it seems that a simpler surface tension scheme than that outlined below could be conceived, for in this case we should have two large porous chambers, communicating by very fine longitudinal capillary tubes with a central reservoir. If the hyaloplasm were in contact with the entire free surface of the porous substance, then lowering the surface tension between the two would tend to cause an increase of their area of contact, i.e., swelling of the base of the sarcomere would occur, hyaloplasm would be drawn in from the central reservoir, through the lowering of pressure as dilatation occurred, and the chief histological features of contraction could be accounted for. But all the histological evidence points to the conclusion that the large porous surface is situated in the middle of the sarcomere, and not at its ends, and this hypothesis need no longer be entertained. This view would further imply that the movement of the hyaloplasm was a secondary consequence of contraction; we have seen above that the opposite is the case.

6. During excitation lactic acid is liberated; we cannot, however, say, *a priori*, whether this acid will cause a decrease or an increase of the surface tensions existing within the fibre, since it may cause a decrease by charging a surface, or an increase by depolarizing a previously charged membrane. I have assumed that the lactic acid is liberated in the interfibrillar spaces, and that it diffuses on to the walls of the sarcomere, exerting its effects, not by coming into direct contact with the active surface, but rather by polarizing the surface through its electrostatic action (9, 10). This theory is in harmony with the conclusion recently reached by A. V. Hill, namely, that lactic acid acts, not by spreading over the active surface, but by altering the degree of polarization of a membrane, and thereby changing the strain on this surface in a manner analagous to the removal of a strain from a condenser on discharging it (5).

Before considering the surface tension mechanism which seems to underlie muscular contraction, a physical analogy may first be discussed. Suppose one end

REFERENCE TO PLATE.

Fig. 1. Portion of uncontracted body muscle of full-grown larva of a beetle (*Cnemoplites blackburni*) in thin longitudinal section (X 2000). The individual sarcomeres are clearly seen. Z indicates Krause's membrane.

Fig. 2. The same, in a contracted state. Note the "reversal" of the striations. (X 2000.)

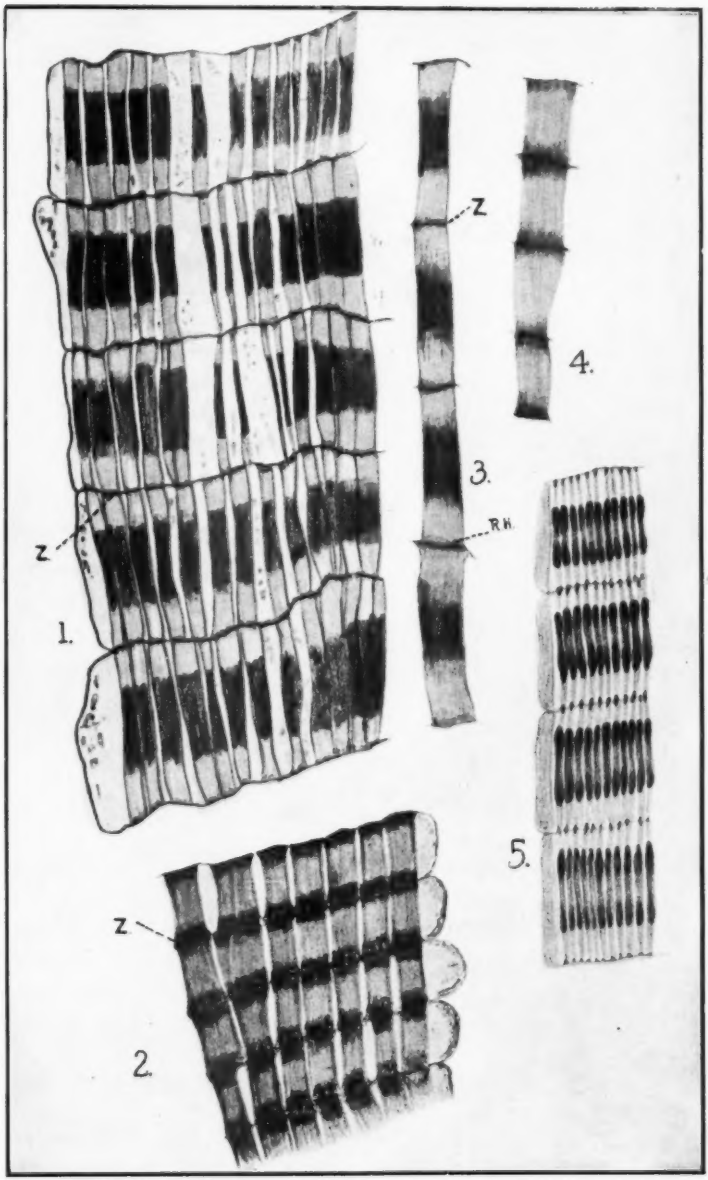
Fig. 3. Portion of a relaxed myofibril from same (X 3400). Z, Krause's membrane; R.H., residual hyaloplasm.

Note that the residual hyaloplasm is connected by very fine streaks of stainable substance with the movable hyaloplasm, which comprises the striation.

Fig. 4. The same, in a contracted state (X 3400). The narrowing of the fibril towards its lower end is due to its being cut tangentially.

Fig. 5. Part of leg muscle of an adult chalcid wasp (*Nasonia*).

Note Hensen's line; also the residual hyaloplasm. (X 2000.)



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of a capillary tube is dipped into a dish of water, and the conditions so arranged that gravity will not prevent water from moving along the tube (figure 6). Consider first the case in which the liquid (water) wets the solid (glass) entirely. When the capillary tube is dipped into the water a film of water spreads over the surface of the glass; the surface tension between the air and the water in the tube tends to reduce this surface to a minimum, and the water is drawn along the tube. Since gravity is not acting upon the water column, the water will eventually be drawn right along the tube. Suppose now that previous to dipping the tube into water a distensible rubber cap (A) had been fitted to its free end; if the tube were now dipped into the water, the water would be drawn into it, until the surface tension force between the air and the water within the tube was equal to the gradually increasing elastic resistance of the rubber cap. Since the total superficial energy of a fluid is proportional to its surface, the degree of distension of the

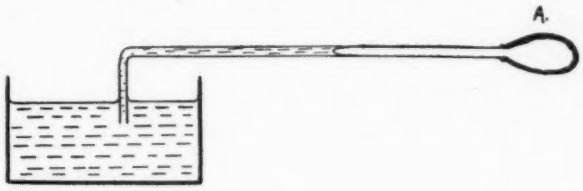

Fig. 6

rubber cap could be greatly increased by making the area of contact between the air and the water in the tube very large, such as by having this region in the form of a spongework, rather than of a simple tube. If the surface tension between the air and the water were now decreased, then the elastic recoil of the rubber cap would overcome the surface tension force, and the water would be forced backwards, until the elastic resistance of the rubber cap was equal to the opposing surface tension force. When the tension was again increased water would again move towards the cap, which would distend. Suppose further that the reservoir of water in the model were replaced by an elastic sac of such a kind that it was distensible only transversely. In this case, if we lower the surface tension between the air and the water, the rubber sac would markedly dilate, while the cap A would tend to collapse; furthermore, the dilatation of the sac would be mainly at right angles to the direction of maximal collapse of the cap.

If we had chosen a liquid that did not completely wet the glass, the results would have been the same, except that the area over which the surface tension force could act would be very considerably smaller.

The above model seems to illustrate well the principle on which the surface tension machine of the muscle acts. The rubber sac represents the spongy tissue at the two ends of the sarcomere, which contains the residual hyaloplasm. The

capillary tube of the model is represented in the sarcomere by the very fine capillary tubes that connect the cavity of the doubly refracting porous material with the loose, spongy tissue at the ends of the sarcomere, and also by the porous material itself. The analogy is incomplete to the extent that the rubber cap in the model becomes in the sarcomere the cavity of the porous material, which at the same time exhibits the large surface area. The hyaloplasm is represented by the water. The air in the model, I previously supposed, was represented by water vapour. I do not believe that this suggestion is a good one, for one does not expect to find free vapours within living tissues. I would here suggest rather that the third surface, which is necessary for the working of the surface tension machine as it seems to occur in musele is presented by a liquid. It may seem that this is an unjustifiable assumption; nevertheless, there is some direct microscopical evidence in its favour. It is often possible to observe, in the middle of the doubly refracting region of the sarcomere, a light weakly staining area (Hensen's line) (see figure 5). In the flying muscles of insects it is indeed extremely prominent. I would suggest that this light staining area in the middle of the porous material is occupied by the third substance, necessary for the working of the surface tension machine. It is clear, at the same time, that if the hyaloplasm entirely wets the walls of the sarcomere, a well-defined pale region in the middle of the dark portion in the relaxed sarcomere might not necessarily be distinctly visible.

The working of the surface tension machine would then be as follows: The contracted sarcomere undergoes relaxation as a result of an increase in the surface tension between the hyaloplasm and the fluid which occupies the region of Hensen's line. The hyaloplasm is drawn further into the doubly refracting porous substance, and since a very considerable free area separates the two fluids in this region, a considerable force, causing a distension of the middle of the sarcomere, will be developed. This will be counteracted by the elastic rebound of the porous material, and a position of equilibrium will be attained. When the relaxed sarcomere is to contract, a fall in the surface tension between the two liquids occurs; the elastic recoil now overcomes the opposing surface tension force, and the fluid is driven out of the porous material, through the fine capillary tubes, into the collapsible sacs or spongework at either end of the sarcomere. If these sacs are of suitable structure, they will thicken from side to side; this will cause the ends of the sarcomere to distend, so that the sarcomere as a whole will shorten.

Concerning the manner in which the lactic acid brings about the surface tension change no definite facts are available, but the following hypothesis may be entertained. It seems improbable that the delicate contractile machine should receive and store the actual fatigue products during its activity; we shall assume that the main chemical reactions that bring about contraction occur in the interfibrillar space.

Now suppose that the wall of the sarcomere constitutes a membrane, semi-permeable in such a way that it will permit diffusion of the small hydrogen and hydroxyl ions, etc., but not of the large ions, such as protein, and perhaps even lactate ion. Let us suppose the hyaloplasm to be of such a nature that if it could

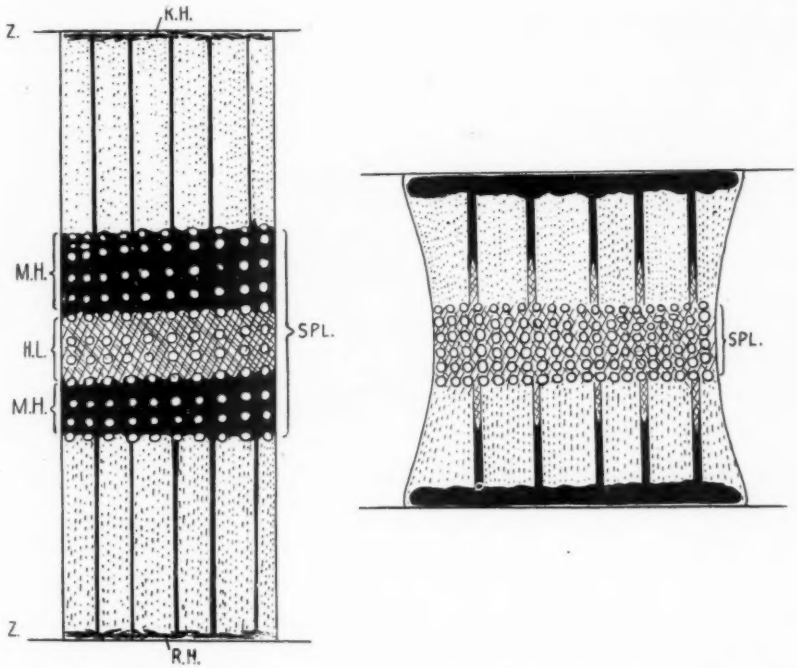

Fig. 7. Diagram of two sarcomeres, in the relaxed and contracted conditions respectively. Z, Krause's membrane; SPL., doubly refracting porous material ("spongioplasm"); H.L., fluid comprising Hensen's line; M.H., movable hyaloplasm; R.H., residual hyaloplasm.

The diagram attempts to show the collapsible spongework adjacent to Krause's membrane; also the stretching of the elastic porous material (SPL.) of the relaxed sarcomere. On the other hand, it fails to show how the surface of contact between the two fluids H.L. and M.L. increases when contraction occurs. Since the fluid M.H. is regarded as completely wetting the porous material, it will extend as a thin film into the porous material, even when the sarcomere is contracted; but it is not possible to represent this in the diagram.

be completely dissociated, it would have a preponderance of negative hydroxyl ions. Since, however, the hydroxyl ion is but weakly dissociated in protein, therefore the interface between the hyaloplasm and the walls of the sarcomere will be but feebly polarized. When free lactic acid is liberated on to the sarcomere the hydrogen ions, diffusing into the thin walls of the sarcomere, will combine with the small quantity of free hydroxyl ion present there. More hydroxyl ion

will be formed and combine with the hydrogen ion. The sarcomere thus presents a condition analogous to a charged condenser; the hyaloplasm will be charged, and this charge will be held by the lactate ions on the outer walls of the sarcomere. If we must conclude that the walls of the sarcomere are too thick to permit the hydrogen ions to reach the hydroxyl ions, then we should have to regard the sarcomere wall as permeable to the lactate ion, and place the semipermeable membrane at the molecular interface between the hyaloplasm and the sarcomere wall.

The charging of the hyaloplasm will result in the lowering of its surface tension, and contraction of the sarcomere will take place, through the action of the surface tension mechanism above discussed.

Meanwhile basic creatine, formed on the second excitable membrane (11), will diffuse on to the walls of the sarcomere, but since its rate of diffusion is much slower than that of the lactic acid (11), it will reach the sarcomere later than will the acid. The hydroxyl ions will enter the walls of the sarcomere; in the interfibrillar space creatine lactate will form, and the condenser will be discharged. The surface tension of the hyaloplasm will again rise, and the sarcomere will once more relax.

The interpretation which I previously placed on the shape of the twitch curve (9) would have to be modified to some extent. The hydrogen ions, suddenly penetrating the membrane of the sarcomere, will explain the sudden rise of the twitch curve; the slower fall would be due to the slower rate of diffusion of the creatine; and the gradual sagging away of the contraction towards its end will be the result of the falling off of the rate of diffusion, as the concentration of the diffusing *base* decreases. The interpretation which I have placed upon the delay in the rate of relaxation that accompanies an isometric twitch carried out under a condition of initial elongation (Hartree and Hill) will in no way be affected; it is due merely to a proportional increase in the longitudinal distance through which the base must flow, as the distance between the two excitable membranes is increased. The theory also offers, as I have previously tried to show (10), an obvious explanation of the fact observed by Heidenhain, Fick, and recently by Fenn (4), that the amount of heat liberated by a muscle when lifting a weight is greater than that liberated when contracting isometrically, the excess heat liberated being an internal frictional effect, capable of being estimated in terms of Poiseuille's formula. Lest it might be urged that Poiseuille's law is valid only for thin capillary tubes, it may be pointed out that Bartell has recently found it to hold even for the case of fluids forced under pressure through a porous filter impregnated with copper ferrocyanide (2). That much of the heat liberated by a contracting muscle during the performance of work is of "thermodynamic" origin has also been suggested by Azuma (1).

On Amoeboid Movement.

Functionally the extrusion of a pseudopod by an amoeboid cell is analogous to the contraction of a sarcomere; in either case an external resistance is overcome. But the resemblance appears to be a much closer one.

The formation of pseudopodia in leucocytes has been described by Schäfer, and illustrated by some beautiful micro-photographs of the living cell (7). The middle region of the cell is occupied by a delicate lace-like reticulum, occupying the region usually described as endoplasm. When a pseudopod is about to be extruded, a granular fluid (ectoplasm) flows out from the reticulum, and the cell wall is caused to protrude in this region. The pseudopod grows in size as the ectoplasm flowing into it increases in quantity; finally the endoplasmic reticulum also enters the pseudopod, but this is clearly a secondary effect. The pseudopod is retracted again by the ectoplasm being withdrawn into the endoplasm.

The analogy to a contracting sarcomere is obviously a close one; the endoplasmic reticulum will correspond to the doubly refracting porous substance of the sarcomere; the granular ectoplasm flowing from the reticulum into the region between the cell wall and the endoplasm is obviously analogous to the movement of hyaloplasm in the sarcomere from the central porous material into the loose spongework at the ends of the sarcomere. The third surface necessary for the working of the surface tension machine would be provided, in an amoeboid cell, by the fluid which pervades the endoplasmic reticulum, after the ectoplasm has been extruded. The presence of this fluid (which would correspond to the fluid forming Hensen's line in the sarcomere) is easily recognizable through the fact that it contains numerous large granules usually undergoing marked Brownian movement.

Underlying the protrusion and retraction of a pseudopod, then, there would be the following processes: When a pseudopod is to be extruded there occurs a lowering of the surface tension between the granular ectoplasm and the fluid content of the endoplasm; and on account of the elastic recoil of the reticulum, the ectoplasm will gradually flow out from the reticulum and will cause a bulging of the cell wall, resulting in the formation of a pseudopod. When the surface tension is again increased, the ectoplasm is once more withdrawn into the reticulum; the surface tension force between the surrounding water, or medium, and the wall of the cell will tend to reduce the area of contact to a minimum, and, the opposing force having been removed, the pseudopod will be obliterated.

In the case of excised muscle this latter force is not acting; which explains the fact that a muscle undergoes only incomplete elongation when contraction ceases, unless an external force be applied to elongate it again.

SUMMARY AND CONCLUSIONS.

1. The unit of contraction of a muscle fibre is the sarcomere.

2. The contractile machine consists of two fluids in contact, and supported within an elastic porous substance, whose cavity communicates by very fine tubes with a collapsible spongework at either end of the sarcomere. When the surface tension between the two fluids is decreased, fluid is forced out of the porous substance, as a result of the elastic recoil of the latter, into the spongework, and the sarcomere thickens at its ends, and therefore shortens; when the tension is again increased, fluid is again drawn into the spongework.

3. The contractile machine works on the same principle as does the capillary electrometer, i.e., a change in the surface tension of a fluid, accompanying a change in its state of electrification. A mechanism is suggested whereby the lactic acid brings about this electrification; neutralization of the lactic acid by basic creatine abolishes the electrification, and the sarcomere again relaxes.

4. The same principle appears to underlie amoeboid movement in general.

REFERENCES.

1. Azuma, R.: Proc. Roy. Soc. (London) (series B), 96 (1924), p. 338.
2. Bartell, F. E.: Journ. Phys. Chem., 15 (1911), p. 659.
3. Bernstein, J.: Pflüger's Arch., 85 (1901), p. 271; *ibid*, 162 (1915), p. 1.
4. Fenn, W. O.: Journ. Physiol., 58 (1923), p. 175.
5. Hill, A. V.: Proc. Roy. Soc. (London) (series B), 98 (1925), p. 506.
6. Robertson, T. B.: Quart. Journ. Exp. Physiol., 2 (1909), p. 304.
7. Schäfer, E. A.: Quart. Journ. Exp. Physiol., 3 (1910), p. 285.
8. Tiegs, O. W.: Trans. Roy. Soc. (South Austr.) 47, (1923), p. 142.
9. Tiegs, O. W.: This Journal, 1 (1924), p. 11.
10. Tiegs, O. W.: *ibid.*, 1 (1924), p. 47.
11. Tiegs, O. W.: *ibid.*, 2 (1925), p. 1.

A NOTE ON THE ENZYMES OF THE HYDATID CYST.

by

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(Submitted for publication 11th December, 1925.)

In a previous paper (3) a study of the micro-chemical reactions of the hydatid cyst wall was described. The next step in the investigation was to determine what enzymes are present in the cyst wall and in the fluid contained within its cavity. For this purpose it has been necessary to make use of two different methods of approach, one suitable for the study of enzymes in tissues, the other for enzymes in fluids. In connection with the former, the auxanographic or plate method, as described by Eijckman (5), working with bacterial enzymes, and by Buxton (1) in his studies on the enzymes of tumours, has been employed. In the latter case the ordinary laboratory methods could be used. The results obtained are only qualitative.

Methods.

(1) *For the Determination of Enzymes in the Cyst Wall.*

A series of freshly prepared agar plates were made in the manner described by Buxton (1), so as to test for diastase, lipase, protease, and oxidase. For detecting diastase, two media were used, one containing 0·5 per cent. starch, the other 0·25 per cent. glycogen. For lipase the media contained 1 per cent. butyrin and 1 per cent. olive oil. For proteases three media were prepared, containing 5 per cent. suspension of egg white, 10 per cent. sterilized milk, and 10 per cent. gelatin respectively.

In testing for oxidases the guaiac and peroxidase tests used by Buxton were employed, and plates containing 1 per cent. hydrokinone were found to be satisfactory, though the indo-phenol reaction could not be used on account of the rapid oxidation of the surface of the plates, which occurred without the addition of oxidase-containing substances.

Organs containing hydatid cysts were obtained from the abattoirs as soon as possible after the animals had been killed, so as to minimize post-mortem change and invasion of the cyst tissue by bacteria. The cysts were removed from these with strict aseptic precautions. All receptacles and instruments were sterilized

in an autoclave, and great care was taken to prevent any contamination. That success was achieved is shown by the fact that in no case was there bacterial growth on the media employed. Many cysts were examined, chiefly from the liver, but also from the lungs, and fluid from both sources was tested separately.

The surface of the organ was first cauterized, and a deep incision made with a sterilized scalpel to expose the cyst, which was opened with a fresh scalpel after the fluid had been aspirated. The mother and daughter cysts were then removed with forceps, cut into very fine pieces with scissors, and ground up in a mortar with fine sand. Glycerin of 50 per cent. strength was added until a soft paste was obtained. Small pieces of this material were placed on the plates, which were incubated at 36°C. for three days and observed every twenty-four hours.

(2) *For the Determination of Enzymes in Hydatid Fluid.*

The methods employed were for the most part those described by Cole (4) in his text-book. In every case freshly prepared reagents were used. For diastase Sladden's modification (6) of Wohlgemuth's method was selected, and also the incubation of relatively large amounts of fluid (15 cc.) with 2 cc. of 0·1 per cent. solution of starch or glycogen, the mixture being titrated with N/10 sodium hydroxide before incubation to a pH of 6·7 using two drops of 1·0 per cent. neutral red as an indicator.

The presence of lipase was determined by incubation at 38°C., with olive oil, in the manner described by Cameron (2), and with butyrin. For proteases, Mett's method, with small tubes of egg white, and 0·4 per cent. hydrochloric acid, as well as incubation of varying quantities (up to 10 c.c.) of hydatid fluid with egg white, coagulated milk, and fibrin as substrates in the presence of 0·4 per cent. solution of sodium hydroxide were used.

Results.

The results of these various tests are presented in tabular form. In every case control experiments in which no hydatid fluid or tissue was used were negative.

	CYST TISSUE.			CYST FLUID.		
	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.
Diastase.						
i. Starch splitting	+	++	++	—	—	—
ii. Glycogen splitting	+	++	++	—	—	—
Lipase.						
i. Acting on Butyrin	—	S.	S.	—	V.S.	V.S.
ii. Acting on olive oil	—	S.	S.	—	V.S.	V.S.
Proteases.						
i. Egg albumen	—	—	—	—	—	—
ii. Milk	+	++	++	—	—	—
iii. Gelatin	—	V.S.	V.S.	—	—	—
Oxidases.						
i. Hydrokinone	—	V.S.	V.S.	—	—	—
ii. Guaiac test	V.S.	—	—	—	—	—
iii. Peroxidase test	V.S.	—	—	—	—	—

+ = reaction. — = no reaction. S = slight reaction. V.S. = very slight reaction.

It is therefore concluded that the chief enzymes found in the hydatid cyst wall are diastatic, that there are in addition a proteolytic enzyme which coagulates milk, a lipase (present only in traces), a protease which liquifies gelatin, and oxidases, all of these being much less in evidence than the former. In the case of the cyst fluid, except for lipase, which is present in exceedingly low concentration, enzymes are absent. The situation of the cyst makes no difference in this respect, although some evidence was obtained that the concentration of the enzymes varied according to the site of development of the parasite, the diastatic ferment being less easily demonstrated in cysts of the lung than in those of the liver.

The chief points of interest, apart from the purely observational nature of this work, are:

1. That the enzymes of the hydatid cyst are confined to the cyst wall.
2. That diastatic enzymes appear to be prominent, although, like most tissues, fat splitting, and at any rate, certain proteolytic ferments, are also present.
3. That hydatid fluid is, as regards enzymes, practically inert.

The first observation is interesting when one considers that the hydatid cyst is extremely common in the liver, one of the great storehouses of glycogen in the body. It is to be presumed that glycogen plays an important part in the nutrition of the parasite, and the variable amount of sugar found in the cyst fluid may result from glycogenolysis within the cyst wall.

The rich supply of glycogen present in the liver may account for the rapid development in that site of scolices into mature cysts. It is possible also that the presence of glycogen may play a part in the development of the parasites in other organs. Should it be possible to culture the parasite in vitro, whether in the form of a cyst or from scolices, an adequate supply of a suitable carbohydrate may prove to be an essential factor.

ACKNOWLEDGMENTS.

I have to gratefully acknowledge assistance from Dr. C. H. Kellaway (Director of the Walter and Eliza Hall Institute of Research), Dr. W. Davies (of the Organic Chemistry School), Mr. A. B. P. Amies (of the Dental Hospital), and Mr. Dickinson (of the Pathological Department at the University).

REFERENCES.

1. Buxton, B. H.: "Enzymes in Tumours," Journ. Med. Res., 4 (1903), p. 356; Buxton, B. H. and Shaffer, P.: "Enzymes in Tumours," Journ. Med. Res., 8 (1904-5), p. 543.

2. Cameron, Gordon: "A Note on the Occurrence of Lipase in the Urine in Two Cases of Acute Pancreatitis with Fat Necrosis," Med. Journ. of Aust. (1923), p. 408.
3. Cameron, Gordon, and Fitzpatrick, A. S.: "The Hydatid Cyst; the Microchemical Reactions of the Hydatid Cyst Wall," Amer. Journ. Path., 1 (1925), p. 227.
4. Cole, S. W.: "Practical Physiological Chemistry, ch. 8 (6th ed.).
5. Eijckman, C.: "Ueber Enzyme bei Bakterien und Schimmelpilzen," Central f. Bakt., 29 (1901), p. 841.
6. Sladden, A. F.: "Some Observations on the Diastase Reaction of the Urine," *Lancet* (1922), p. 68.

STUDIES ON THE REFRIGERATION OF MEAT.

I. INVESTIGATIONS INTO THE REFRIGERATION OF BEEF.

by

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1. INTRODUCTION.

It is well known that beef which has been frozen slowly in air and then thawed loses from all cut surfaces a clear, reddish-coloured fluid. This exudation, known as "drip," contains about 9% protein, and its presence shows that the cycle of the changes of phase of the beef, from liquid to solid and back to liquid, produces definite alterations in the tissue structure, whereby water and protein are liberated. The work of the British Food Investigation Board (1), and of the German workers Plank and Kallert (11), has shown that the presence of drip is due to changes which have taken place during the freezing, and which are not reversed when the material is thawed. The same workers also showed that one of the factors affecting this change is the rate of freezing, and that much of the drip may be prevented by freezing at a sufficiently rapid rate. The British Board (2) have investigated the effects of different rates of freezing upon simple colloids, particularly protein sols and gels. They found that when a 12% gelatin gel was frozen slowly, for instance in air at —11°C., water separated from the colloid, froze as ice on the outside of the system, and was never completely reabsorbed on thawing. The residual colloidal mass was thus "dehydrated," and contained, in some cases, as much as 54% gelatin. When freezing was carried out more rapidly, ice crystals formed within the gel, and the quantity of water which separated when the mass was thawed was greatly lessened. When the gels were frozen in liquid air, the rate became so great that the whole froze as a "glass," and on thawing the material differed from the original gel only in being slightly more opaque. The general conclusion was that the less the amount of water separating out during the freezing, the greater was the degree of reversibility on thawing.

It was further found that a great deal of the water which had separated from the gels during slow freezing was reabsorbed by the gel subsequent to

thawing, but this absorption was never complete. The freezing had apparently caused a loosening of the union between the dispersed phase and the water, and the resulting "dehydrated" mass was capable of imbibing water to a limited extent. The rate of this reabsorption, however, could be increased by raising the temperature, so that in the cases of hydrosols and hydrogels of protein it might be possible to obtain complete reversibility by a combination of rapid freezing and slow thawing, and a subsequent exposure to a temperature above 0°C.

More recent work, however, seems to show that there are other factors which operate in the change, one of which may be the actual temperature to which the colloid is cooled during the process. Thus Moran (8) states that when hens' eggs are frozen by exposure to temperatures above —6°C., complete reconstitution of both yolk and white takes place on thawing. If, however, the temperatures employed be below —6°C., or even if the eggs be frozen above —6°C., and later stored at —11°C., an irreversible change takes place, so that after thawing, the eggs have pasty yolks and turbid whites. In a review of the theories of the freezing of tissues, Plank (9) suggests that there may also be a similar critical temperature with muscle colloids. In a later paper (10), he points out that at the freezing point of muscle (approximately —1·5°C.) only 42% of the water is frozen, that a fall of temperature below this point causes a gradual separation of the unfrozen water from the colloidal mass, and that after thawing such water is reabsorbed only with difficulty. Consequently exposure to low temperatures would tend to produce an irreversible change.

The problem of refrigeration of meat is therefore a very complex one. Ox muscle may be regarded as consisting of a number of colloidal systems, each composed of the muscle plasma, bounded by a semi-permeable membrane (the sarcolemma); any theory of the freezing of tissues must take into account changes in both these elements during freezing and thawing.

The present paper records experiments carried out for the Committee for the Freezing of Beef* of the Australian National Research Council. The object of the investigation was to develop, if possible, such methods of treatment that the beef underwent no changes during the freezing process which could not be reversed during thawing. In addition, it was sought to obtain data on the rates of freezing and thawing which could be attained with pieces of material similar in size to those actually used by the trade (e.g., quarters of beef). This latter work was carried out at the suggestion of the British Food Investigation Board, which at that time had more or less confined its attention to the investigation of the freezing and thawing properties of small pieces.

The actual biochemical and histological work has been carried out by J. R. V. and the physical measurements by G. A. C.

* It was at the suggestion of the chairman of this committee, Dr. J. A. Gilruth, that the investigation in Australia was commenced.

2. METHODS OF ATTACK.

(a) Methods of Freezing and Thawing.

In these investigations two methods were employed to vary the rate of freezing. In the first method, media at different temperatures and with different specific conductivities were employed, such as cold air and a calcium chloride ("brine") bath. Similar samples could thus be frozen at different rates, and when subsequently thawed under similar conditions, gave the same rates of thawing. By this method, however, the more rapidly frozen pieces were reduced to a lower temperature in the process than the others. As very low temperatures are stated to be one factor in changes occurring during freezing, this would tend to increase changes in the more rapidly frozen samples, and hence to lessen any differences between these and the more slowly frozen samples. This influence was avoided in the second method by employing the same freezing bath and varying the size of the samples. Pieces of the meat were cut exactly to fit rectangular tin containers of the same length and breadth, but varying in thickness. When immersed in the same bath these gave a series with gradually increasing rates of freezing. This method, however, introduced other differences, in that when the pieces were thawed under similar conditions, the time taken in thawing also varied with the thickness. In this case also, as will be seen later, slow thawing would tend to bring about reconstitution, and therefore the differences in thawing would again lessen any changes due to slower freezing. In both methods, therefore, the differences actually observed would be smaller than the changes due to the rate of freezing alone.

For the extreme rate of freezing, liquid air was employed. Different thawing rates were obtained by employing air or water as the medium.

(b) Measurement of Temperature.

It was essential in these experiments to have an accurate method of estimating the rates of freezing and thawing. In the earlier experiments, following the practice of the British Food Investigation Board (6), copper constantan thermocouples were employed, the individual wires of which were insulated with either shellac or celluloid, and the junctions enclosed in about a foot of glass barometer tubing of five-sixteenths of an inch external diameter. The cold junctions were immersed in a mixture of distilled water and powdered ice contained in a vacuum flask. The recording instrument was a millivoltmeter of the moving coil type made by Cambridge and Paul, and the couples were of sufficient length always to allow of the millivoltmeter being placed outside the cold chamber. All thermocouples were calibrated periodically by immersing them in (a) melting mercury and (b) in brine at various temperatures, and in which was also placed a standardized mercury-in-glass thermometer graduated into tenths of a degree.

When dealing with the larger portions of meat, e.g., hindquarters, couples were inserted at different depths, and for this purpose glass-covered couples were found to be more convenient. While it was recognized that the glass covering would be conducive to lag in the temperature recorded, it was found by comparison with bare couples that such lag was small, and in the larger portions, where freezing was a matter of hours, relatively unimportant. At a later stage of the investigations, however, when small portions were under examination, lag became of much more consequence. In order to obviate it, a bare couple with an insulation of rubber vulcanized in situ devised by Love (7) was employed. Trials of this couple showed that its lag was less than 30 seconds and that its insulation was more robust and more satisfactory than the shellac and celluloid previously used.

In each experiment the temperature was read at frequent intervals and plotted against the time. The curves so obtained were of the general form reported by the British workers (1). During freezing they flattened when a temperature of —1·5°C. had been reached, corresponding to a temperature at which a very considerable proportion of the water content of the meat solidified. From —1·5°C. the temperature fell slowly to about —3°C., after which the fall became more rapid. The curve obtained during the thawing was flattened in a similar manner. The period of time corresponding to this distinct flattening of the freezing and thawing curves is referred to as the "latent period" of freezing or thawing, as the case may be. It gives a measure of the rapidity with which freezing or thawing takes place, irrespective of the thickness of the meat or the temperature of the medium in which it is placed. In view of the many different conditions of freezing and thawing employed, this use of "latent period" was considered more suitable for purposes of comparison than a system based on the time taken for the material to pass from, say, 5°C. to —5°C.

The rates of freezing quoted throughout this paper are those found at the deepest part of the piece, i.e., the maximum latent period. No attempt was made to obtain figures when liquid air was used as the freezing medium.

(c) Estimation of "Drip."

After considerable experiment, a method was devised of obtaining an accurate comparison of the amount of "drip" by absorbing in blotting paper. Uniform slices of the thawed meat were cut about 3 mm. in thickness, they were weighed, placed between sheets of nitrogen-free, absorbent paper, and a small uniform weight placed on top to keep the meat in contact with the paper. The whole was covered by a box to reduce evaporation as much as possible. Two hours were found ample time to absorb the drip. The loss of weight of the beef was then determined, and the drip was calculated as a percentage of the weight of the beef. "Blank" drip experiments were always carried out on the fresh sample before

treatment, and the difference between this value and the value for the thawed meat was called the "nett" drip. This method gave fairly consistent results; thus, in one experiment five pieces cut from the same position in the buttock gave respectively 5·45, 5.24, 5·50, 5·42, and 5·85 per cent. of drip, and in another three pieces from a rib gave 6·25, 6·50, and 6·20 per cent.

In order to obtain reliable estimations, at least three determinations were made at each position with each piece of beef under investigation.

(d) Determination of the Nitrogen Content of the "Drip."

From this determination could be calculated the approximate percentage of protein present in the drip. These determinations were carried out on the drip absorbed on the paper, by digesting the latter with sulphuric acid and estimating the nitrogen by Kjeldahl's method in the usual way.

The nitrogen loss due to irreversibility of the beef during the freezing and thawing cycle was determined by subtracting the nitrogen content of a "blank" determination on the fresh meat, from the nitrogen content of the drip obtained from the frozen and thawed meat. This nitrogen loss is expressed as a percentage of the total drip. Although it is not strictly correct to speak of this difference as the nitrogen loss, it gives a convenient method of comparison between different types of beef where blank drip contains different quantities of protein.

(e) Histological Investigations.

Microscopical examinations were made by cutting very thin sections in the frozen condition and examining them whilst still frozen. In addition, ordinary histological preparations were made after the beef had been thawed. In other experiments small pieces of frozen tissue were fixed before thawing by dropping into 10% formalin, which had been cooled to its freezing point, and sections were prepared in the usual manner. Since much distortion of structure may be caused during the usual histological preparation, more reliance was placed on the microscopical examination in the frozen state. Except where otherwise stated, the histological examinations were carried out on transverse sections from the deepest part of the frozen muscle, thus obtaining the position of maximum distortion.

As it was found that the size of the ice crystals increased concurrently with an increase in the time of freezing, measurements of the average diameters of numbers of approximately circular-shaped crystals were made. This method of measuring the amount of distortion of normal structure due to freezing, though only approximate, gave results upon which conclusions could be based. It was frequently necessary to distinguish between ice crystals and muscle fibres. For this purpose a petrological microscope was used, and thin sections were examined

between crossed nicols. In contra-distinction to the ice crystals, the solid fibres did not extinguish uniformly, but usually showed several alternate rings of black and white.

(f) Autolysis.

Autolysis of the muscle tissue was examined by incubating the minced tissue at a definite temperature (35°C.), and estimating the percentage of the total nitrogen not precipitated by 10% tannic acid. This method is very similar to that employed by Fearon and Foster (4), except that they employed trichloracetic acid in place of tannic acid as precipitant. These workers found that by plotting the percentages of unprecipitated nitrogen against the time, in the case of fresh muscle a regular logarithmic curve was obtained, whereas with meat which had been frozen the curve became steeper, this variation increasing with the time taken in freezing. A similar result was obtained by Callow (3) with codfish muscle. These results have been explained as being probably due to the disturbance of the structure of the tissue in freezing, whereby the conditions formed on thawing become more favourable for the action of the tissue enzymes. Therefore it was considered that experiments on the rates of autolysis would yield very valuable information as to the extent of alteration of the normal structure of muscle caused by freezing.

3. EFFECTS OF RATE OF FREEZING.

Throughout these investigations, in each series quoted, beef from the same beast was employed, cut from adjacent positions on the carcase. In every case also full data were recorded as to the time between killing and freezing, age of the beast, and type of meat.

(a) On Drip and Nitrogen Content of Drip.

Table 1 shows the results of a number of experiments on pieces of a commercial size (the buttock being in the thickest portion of a side of beef), and on small pieces of standard size cut to fit the containers. In this table only those series are given which have been thawed under similar conditions, and unless otherwise stated the amount of drip and its nitrogen content refer to that from the position in the piece where it is likely to be greatest, i.e., at the position of maximum latent period of freezing. In all experiments the thawing was carried out in air.

It is seen that in every series of experiments the amount of drip steadily increases with increasing latent periods of freezing. When different series are compared there is, however, in general, very little uniformity between the quantity of drip and the latent period of freezing. As will be seen later, the type and age

of the beast are of great importance in determining the degree of reconstitution of the tissue after freezing and thawing, e.g., experiments 16 and 20.

The only experiment (experiment 5) in which there was no drip was that in which liquid air was employed, and freezing was practically instantaneous. In all other experiments, even when the latent period was reduced to much less than one hour, quite an appreciable quantity of drip was found.

TABLE 1.

Expt.	Age and description.	Thickness Cm.	Medium.	Freezing. Temp. degrees C.	Latent period hours.	Thawing. Temp. deg. C.	Latent period hours.	Nett drip. %	Nitrogen loss. %
1	4 year ox buttocks	26	$CaCl_2Aq$	—36	2·1	12	40	Av. 2·2 Max. 3·1	1·02 0·93
2	„	26	Air	—10	5·5	12	40	Av. 3·7 Max. 5·4	1·10 1·17
3	—	6·5	$CaCl_2Aq$	—28	A few minutes	15	8·5	1·5	0·14
4	—	—	$CaCl_2Aq$	—12	0·75	15	8·5	3·0	1·00
5	—	4	Liq. Air	—193	A few seconds	15	—	—0·1	0·12
6	—	4	Air	—10	10	15	—	2·5	0·26
7	6 year ox pieces	4·5	$CaCl_2Aq$	—18	0·5	5	18	0·7	0·30
8	„	6·25	$CaCl_2Aq$	—18	0·75	5	22	3·6	0·35
9	6 year ox pieces	3·0	$CaCl_2Aq$	—18	0·4	19	1·75	2·9	0·25
10	„	4·5	$CaCl_2Aq$	—18	0·5	19	2·25	3·7	0·45
11	„	7·5	$CaCl_2Aq$	—18	1·1	19	5·0	7·8	0·47
12	„	8·5	$CaCl_2Aq$	—18	1·7	19	6·0	5·5	0·26
13	aged ox pieces	4·8	$CaCl_2Aq$	—10·5	1·55	6	21	3·6	0·30
14	„	6·7	$CaCl_2Aq$	—10·5	2·30	6	36	3·7	0·38
15	„	8·5	$CaCl_2Aq$	—10·5	4·00	6	39	4·3	0·47
16	4-5 year ox pieces	3·3	$CaCl_2Aq$	—14	0·6	1	25	1·4	0·26
17	„	5·0	$CaCl_2Aq$	—14	1·1	1	50	1·5	0·37
18	„	7·0	$CaCl_2Aq$	—14	2·2	1	64	3·4	0·13
19	„	9·5	$CaCl_2Aq$	—14	5·0 (?)	1	96	3·9	0·16
20	very aged ox pieces	3·3	$CaCl_2Aq$	—23 to —27	0·18 0·55	0·6 0·6 to ·20	65 64	3·1 4·4	0·15 0·19
21	„	5·0	$CaCl_2Aq$	—23 to —27					
22	„	7·0	$CaCl_2Aq$	—23 to —27	1·70	0·6 to ·20	88	6·6	0·19
23	„	10·0	$CaCl_2Aq$	—23 to —27	2·30	0·6 to ·20	99	7·4	0·28

The nitrogen loss figures show that the protein loss usually ran parallel with the increase in latent period, but there was not the same regularity as with the drip.

(b) Histological Structure.

Photomicrographs of frozen sections were taken (see plates, figures 1-13), but it proved very difficult to cut sections which contained ice, and which were

sufficiently thin and uniform to eliminate the effect of depth, and consequently some of the sections are slightly out of focus in several places.

Figure 1 represents a section of beef frozen by means of liquid air. In this case, owing to the opacity of the section, individual fibres can only be distinguished with difficulty, but no ice crystal formation is visible.

Figures 2-7 represent a series in Table 1, experiments 9-12, and show a gradual increase of distortion of the tissue with increasing latent period of freezing.

Experiment 9 (latent period of freezing 0·4 hour) (figure 2) shows an unaltered structure as compared with that of fresh beef, save for the beginning of ice crystal formation in a few places, such as at B. The fibres are regularly dispersed, and of uniform polygonal shape, as in a normal section. It is evident that this figure illustrates the beginning of definite distortion, since there is a very small amount of ice formation outside the fibres, and in several places the latter appear to have become compressed, owing to the small amount of ice crystallized around them, forcing them together. Figure 3 is from the same piece, but has been fixed whilst in the frozen condition. Its structure is very similar to that of the frozen section, except that in a large number of the fibres there appears a small hole, as at A, which in the frozen condition has probably been a rod of ice inside the fibre. This demonstrates that the alteration of the normal colloidal structure can definitely take place not only outside, but also inside the fibre, and that the two types of distortion can go on simultaneously. Also since this experiment shows a much greater amount of ice formation within the individual fibre than without, it would appear that the alteration of structure due to freezing commences within the fibre itself.

Figures 4 and 5 are sections, the one in the frozen state and the other after fixing and staining, of the beef frozen in experiment 10 (latent period 0·5 hour). They show that with a slight increase in the time of freezing the alteration of the normal structure has increased. It is difficult to distinguish the ice crystals in figure 4, but figure 5 definitely shows that ice has formed, as at A, in small numerous crystals outside the fibres. The amount of ice formation is, however, not great. In most cases the muscle fibres are regular, but many have been laminated by the pressure of the ice crystals. The freezing has evidently been slow enough to allow of much water separation from the plasma, and its passage into the surrounding medium, since many of the fibres are very small, and the number which show cavities left by ice crystals inside are fewer than in experiment 9.

Experiment 11 (latent period 0·75 hour), figure 6 shows a further stage in the distortion of structure. The amount of ice formation, as at A, and the size of the ice crystals, show that the "dehydration" of the fibres has had time to proceed further. From the presence of so many intact fibres, as at B, it is evident that the freezing has been fast enough to prevent a large percentage of the fibres from becoming both "dehydrated" and distorted by the pressure of the

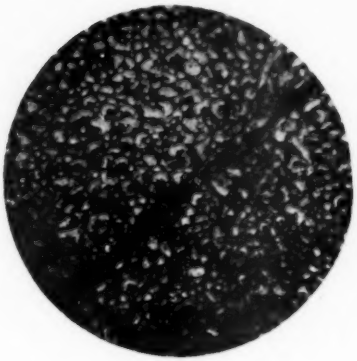


Fig. 1.

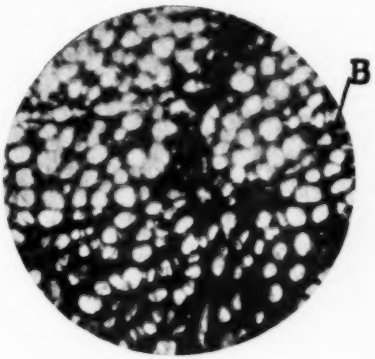


Fig. 2.

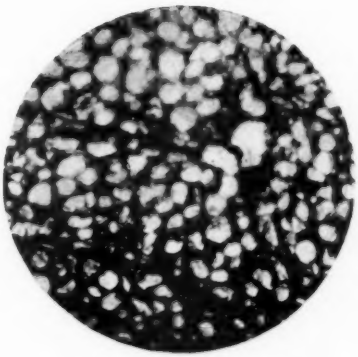


Fig. 4.

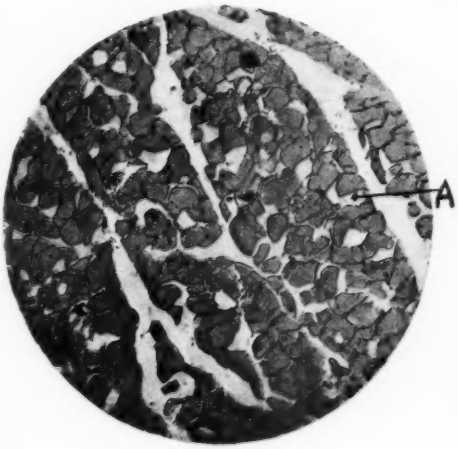


Fig. 5.

Fig. 3.

PLATE 1. SECTIONS OF FROZEN BEEF.

Figs. 1, 2, 4 show sections in the frozen condition.

Figs. 3, 5 show sections which have been fixed whilst frozen, and stained with eosin and haematoxylin.

ice crystals. In this piece also another result of distortion appears, viz., the forcing together of muscle fibres, connective tissue, etc., into a compressed mass by two or more sets of ice crystals which have grown towards one another. Such bands of compressed matter may be seen at C. That this compressed mass has lost a large percentage of its original water content may be judged from the fact that the thin bands are very dense and yellow in colour by transmitted light, and quite unlike the clear or whitish appearance of the undistorted frozen muscle fibres.

Whereas in the last experiment the ice crystals occupy an area of about one-third of the cross section, in figure 7, experiment 12 (latent period 1·7 hours) they occupy at least one-half. It is also seen that the bands of compressed material are more numerous and longer and wider than before.

Similar sections were obtained from other experiments on small pieces of beef recorded in Table 1. It was generally observed that as the latent period of freezing increased the degree of distortion became more marked. There was, however, a certain want of uniformity in the degree of distortion produced in samples of different origin, which differences were probably due to the different ages of the beasts, and also possibly to being reduced to different final temperatures during the freezing. In no case, however, was the original structure preserved after freezing.

Figures 8, 9, and 10 refer to experiments in which pieces were frozen in brine and air, latent periods of freezing being 2 and 10 hours respectively. Figure 8 illustrates the structure of the piece frozen in brine; figure 9 a section of the air-frozen piece in the frozen condition; and figure 10 the same after fixing.

In figure 8 the ice occupies about one-third of the total area, occurring mainly as small isolated crystals. In figure 9, however, it occupies nearly one-half of the total area, and the crystals are relatively large (up to 0·4 mm. width), as may be seen at A in both figures. Another striking point of difference is that there is quite a considerable number of isolated intact fibres present in figure 8, whilst in figures 9 and 10 there are very few. In the former case a certain number only of "dehydrated" and distorted fibres are collected into compressed bands, whereas in the latter almost all the fibres are so arranged (B). These are usually of much smaller area in cross-section, and are laminated in the direction of the compressing ice crystals.

Figures 11 and 12 are taken from the large scale pieces (buttocks) in experiments 1 and 2, Table 1. As it was found impracticable to obtain sections from the centre of the buttocks, wedge-shaped pieces were cut from similar positions in each, and sections were made from these. It was estimated that the approximate latent periods of freezing of these sections were 1 and 20 hours respectively. The difference in appearance is very striking. In figure 11, although the uniform structure has been altered, the number of undistorted fibres is fairly large, the

size of the bands of compressed fibres very small, and the quantity of ice formation not extensive. In figure 12 all resemblance to the structure of mammalian muscle has disappeared. It shows large ice crystals and numerous bands of compressed fibres, which have lost so much water that they appear as dense opaque masses, even in the thinnest sections. The jagged appearance of the ice crystals is due to the difficulty of cutting material containing so much ice without causing the surface of the crystals to become uneven. In no case was such a rate of freezing attained with large pieces of beef that alteration of tissue structure disappeared, the structures presented in the sections from experiment 1 showing the least amount of distortion of any of these experiments.

An attempt was made to compare the size of the crystals of ice. A rough method was employed, which consisted in estimating the average diameters of all ice crystals approximately circular or elliptical in cross-section. A micrometer eye-piece of the rotating type was used capable of measuring to at least $\pm 0{\cdot}2\mu$, and in each case the average was obtained from measurements of twenty-five to thirty crystals.

The figures for the experiments of Table 1 are collected in Table 2, and, with one exception, show an increase in the size of the ice crystals with increasing latent period of freezing.

TABLE 2.

Experiment.	Latent period of freezing hours.	Average diameter of ice crystals.
1	2.1	117
2	55	231
7	0.5	101
8	0.75	134
12	1.70	175
13	1.55	169
14	2.30	159
15	4.00	204
16	0.6	128
17	1.1	144
18	2.2	145
19	5.0	176
20	0.18	109
21	0.55	116
22	1.70	139
23	2.30	152

The general results of the histological investigation show that the only case in which a normal structure was retained after subjecting the tissue to freezing

PLATE 2. SECTIONS OF FROZEN BEEF.

Figs. 6, 7, 8, 9 show sections in the frozen condition.
Fig. 10 shows a section which has been fixed whilst frozen and stained with eosin and haematoxylin.

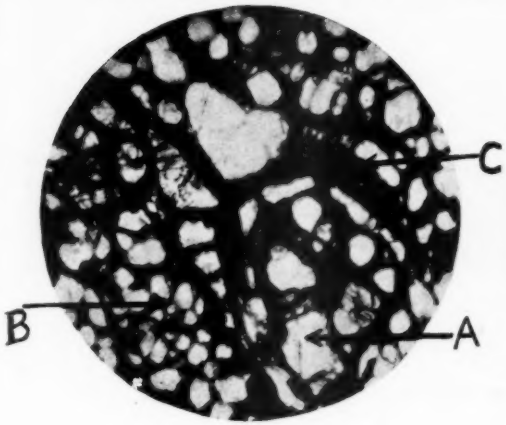


Fig. 6.

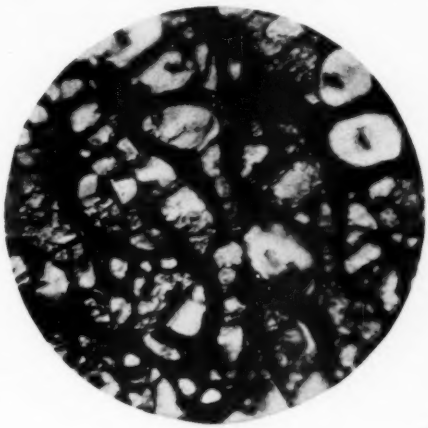


Fig. 7.

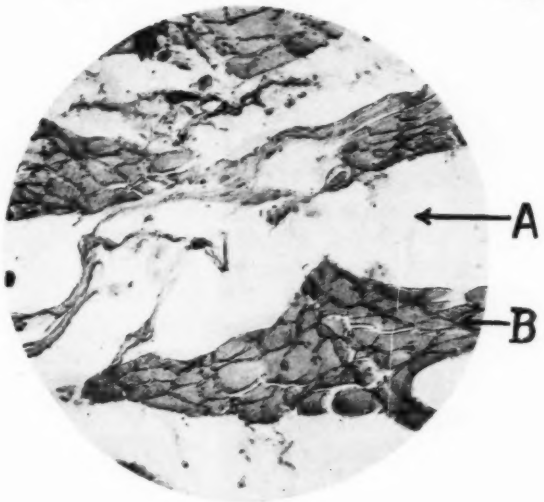


Fig. 10.

Fig. 8.

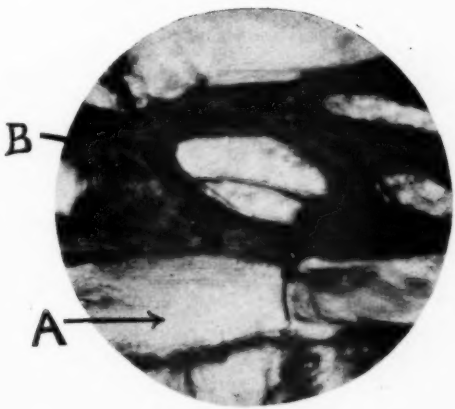
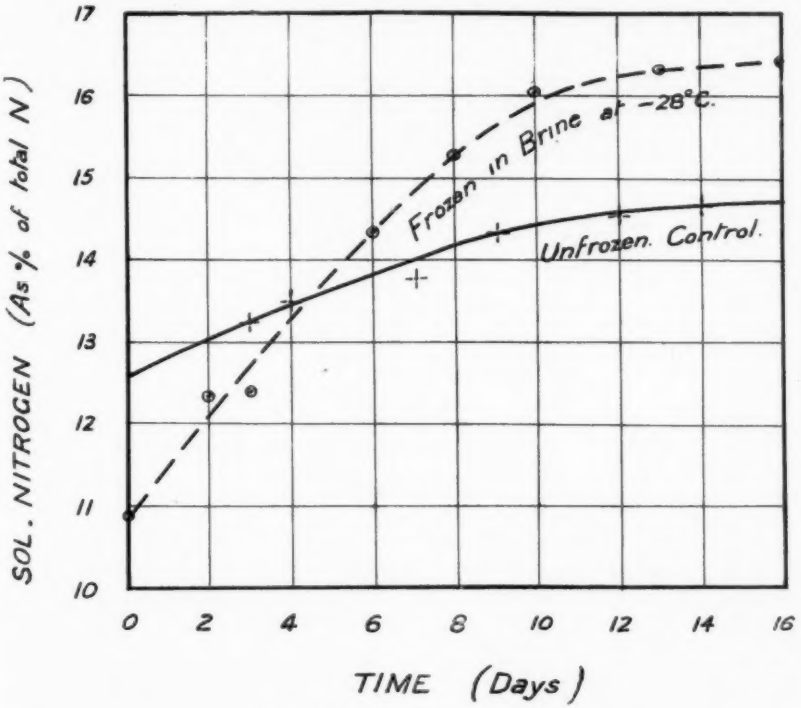


Fig. 9.

was in the piece frozen almost instantaneously in liquid air. The structure most closely resembling this was that shown in figure 9, latent period 0·4 hour, where alteration of the normal colloidal condition had proceeded to some extent inside the fibre, but where there was very little ice formation outside. In all the other pieces, even that (experiment 20) having a latent period of only 0·18 hours, definite distortion of structure was seen. These results were borne out by the figures for the measurement of the ice crystals.



Autolysis of Beef at 35° C.

Two other phenomena which accompany an increase in the latent period of freezing are the gradual lessening of the numbers of distorted, clear fibres, and the increase in the width and length of the bands of compressed "dehydrated" fibres.

(c) Rate of Autolysis.

A typical experiment on the comparative rates of Autolysis in fresh and treated beef is shown in the accompanying curves (A and B). A was obtained

from a sample from a buttock which had been frozen in brine at —25°C. It was cut from a position at a depth of 6 cm., and had a latent period of 1½ hours. B was from the control with unfrozen beef from the same buttock.

It will be noticed that the forms of these autolysis curves are very different. In the case of the frozen beef, both the rate and extent of autolysis is greater. The sample which has been frozen has an initially lower percentage of soluble nitrogen than the fresh meat, usually amounting to about 2%. No explanation has been found for this lowering of the soluble nitrogen content. As suggested by Fearon and Foster (4), the differences in the forms of the autolysis curves are probably due to an alteration produced in the colloidal structure by freezing, which causes it to be more easily hydrolyzed by the autolytic enzymes of the tissue.

4. EFFECT ON THE RATE OF THAWING.

(a) On Drip and Nitrogen Content of Drip.

Previous experiments on the thawing of frozen gels (see introduction) had shown that a considerable quantity of water which had separated during freezing was reabsorbed when the frozen gel was thawed slowly. It might be expected, therefore, that water which had separated from the colloids of beef tissue during freezing might also be reabsorbed, and the amount of drip consequently reduced, if a slow rate of thawing were employed.

As the result of histological examinations, Kallert (5, 6), and Richardson and Scherubel (12), have stated that frozen meat after slow thawing exhibits less change than after rapid thawing. These workers, however, made no quantitative observations on the amount of drip produced, so that in the present investigation it was thought advisable to carry out such determinations, using different rates of thawing. As before, experiments were carried out both on small pieces of beef and on whole buttocks, and in each comparative series the freezing was carried out in exactly the same manner. The drip was estimated as soon as possible after thawing, so as to eliminate the possibility of subsequent absorption. The results are given in Table 3, the freezing medium being calcium chloride solution throughout.

TABLE 3.

			Freezing.			Thawing.			
Expt.	Age & description.	Thickness. Cm.	Temp. deg. C.	Latent period hours.	Medium	Temp. deg. C.	Latent period hours.	Nett drip. %	Nitrogen loss. %
7	6 year ox	4.5	—18	0.5	air	5	18.0	0.7	0.30
10	pieces	4.5	—18	0.5	air	19	2.25	3.7	0.45
24	aged ox	8.0	—15	2.0	air	0.6	90	0.8	0.08
25	pieces	8.0	—15	2.0	air	20.0	4	1.6	0.56
26	aged cow	21	—25	2.8	air	10	54	1.7	0.21
27	buttocks	21	—25	2.8	water	15	14 (?)	3.4	0.47

Fig. 11.

Fig. 12.

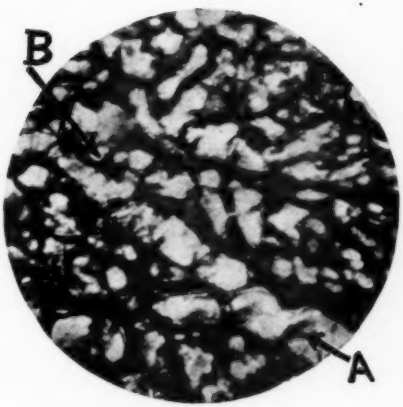


Fig. 13.

PLATE 3. SECTIONS OF FROZEN BEEF AND MUTTON.

Figs. 11, 12, 13 show sections in the frozen condition.

These experiments show that slow thawing had reduced the drip, but that in no case had the drip been completely eliminated.

5. EFFECT OF STORAGE AFTER THE COMPLETION OF THAWING.

Experiments were also performed to ascertain whether the tissue, after thawing had been completed, would still continue to absorb the water separated during freezing. These experiments were carried out by storing the pieces at a temperature above their freezing point, viz., at 1°C. A series of such experiments is given in Table 4. The material used throughout was aged ox, and the freezing medium was calcium chloride solution. The thawing temperature varied from 1° to 20°C.

TABLE 4.

Expt.	Time of storage at 1° C. after thawing. days.	Thickness. Cm.	Freezing. Temp. deg. C.	Freezing. Latent period hours.	Thawing. Latent period hours.	Nett drip. %	Nett nitrogen loss. %
20	0	3.3	−23 to −27	0.18	65	3.1	0.15
28	7	3.3	−23 to −27	0.18	64	5.1	0.17
21	0	5.0	−23 to −27	0.55	64	4.4	0.19
29	7	5.0	−23 to −27	0.55	65	3.3	0.10
22	0	7.0	−23 to −27	1.70	88	6.6	0.19
30	7	7.0	−23 to −27	1.70	88	5.7	0.16
23	0	10.0	−23 to −27	2.30	99	7.4	0.28
31	7	10.0	−23 to −27	2.30	99	3.2	0.11

In three cases out of four, there was thus less drip after storage than immediately after the completion of thawing.

6. EFFECT OF STORAGE AT 1°C. BEFORE FREEZING.

As it had been pointed out in correspondence with the British Food Investigation Board that the osmotic pressure of the contents of the muscle fibres rises steadily after the death of the animal, experiments were carried out to see whether this rise of osmotic pressure had any effect on the amount of drip liberated from a muscle tissue on thawing. It was believed possible that this rise might facilitate in the imbibition by the fibres during thawing of the water liberated outside them.

In order to test this suggestion two series of experiments were carried out by comparing the amount of drip and its nitrogen content from pieces of beef of equal thickness cut from the same carcase, and subjected to the same conditions of freezing and thawing, except that each piece had been stored at a temperature of 1°C. for a variable length of time before freezing. In this way a series of

seven similar samples could be frozen with a definite interval of time between each freezing. In the second set of experiments the rate of autolysis (and perhaps putrefaction) of the stored beef was followed in order to determine whether any extensive changes had taken place in the chemical composition of the beef. In this series the first piece to be frozen was a cut from a beast immediately after killing. Every effort was made to prevent putrefaction from taking place in the stored samples by sterilizing the containers and the sheet rubber coverings which were placed on each container to prevent drying out of the tissue.

The results are given in Table 5, the freezing and thawing media being calcium chloride and air respectively.

TABLE 5.

Expt.	Length of time between killing and freezing.	Age and description.	Thickness. Cm.	Freezing. Temp. deg. C.	Freezing. Latent period hours.	Thawing. Temp. deg. C.	Thawing. Latent period hours.	Nett drip %	Nett nitrogen loss %
17 (a)	4 days	5 year ox	5.0	—12.5	1.2	1	55	1.5	0.37
17 (b)	11 days	5 year ox	5.0	—12.5	1.2	1	55	0.9	0.11
17 (c)	18 days	5 year ox	5.0	—12.5	1.2	1	55	2.95	0.16
32	2 hours	aged bull	5.0	—17	1.0	4	15	5.5	—
33	5 days	aged bull	5.0	—17	1.0	4	15	4.7	—
34	10 days	aged bull	5.0	—17	1.0	4	15	2.8	0.71
35	15 days	aged bull	5.0	—17	1.0	4	15	4.9	0.71
36	20 days	aged bull	5.0	—17	1.0	4	15	2.2	0.85

The results given in Table 5 show that up to the end of ten or eleven days' storage at a temperature of 1°C. before freezing, there is a definite decrease in the drip from the beef after freezing and thawing. When stored for a longer period a slight increase is observed. Experiments carried out on the extent of autolysis show only a slight increase (approximately 10%) of unprecipitated nitrogen, even after twenty days' storage.

7. EFFECT OF AGE OF MUSCLE.

The experiments already given in Table 1 show that generally speaking there was much less drip with the muscle tissue of younger animals than with that of older beasts, and the histological examination also showed much less ice formation, and in consequence less distortion of structure than with the older muscle. Table 6 gives the results of experiments bearing directly on this question, carried out by freezing beef of different ages, each piece being subjected to the same conditions of freezing and thawing. The samples were frozen in calcium chloride solution at —10·5°C., in containers of 4·8 cm. width, and were thawed in air at 6°C. The latent periods of freezing and thawing were 1½ hours and 21 hours respectively.

TABLE 6.

Experiment.	Age and description.	Nett drip, %	Nett nitrogen loss, %
13 (a)	1½ years ox	2.6	0.33
13 (b)	4 years ox	3.5	0.33
13 (c)	very aged ox	3.6	0.30

The figures show a lower amount of drip in the younger muscle than in the older.

Histological examination showed that there was a moderate amount of structural alteration in each tissue, but that the size and amount of ice crystals which had separated out was less in the youngest than in the four years old and the aged muscle.

8. COMPARISON OF BEEF AND MUTTON.

Mutton differs from beef in that when it is frozen on the commercial scale in air at —14°C. ("sharp" freezing), almost complete reversibility takes place on thawing. It was therefore thought possible that a comparison of the changes in beef and mutton brought about by freezing might yield information as to the irreversibility of beef on slow freezing.

Two similar samples of mutton were frozen, the one in liquid air (latent period of a few seconds), and the other in air at —14°C. (latent period approximately 10 hours). Sections were taken from the deepest parts and examined in the frozen condition. Owing to the opacity of the former the cutting of suitable sections proved very difficult, but no distortion of the normal structure could be observed. A section cut from the slowly frozen piece is seen in figure 13. There is considerable ice crystal formation, but most of the crystals are small (A), whilst many intact, undistorted fibres may be seen (B). It is interesting to compare this with sections of beef frozen with approximately the same latent period (figures 9 and 10). The differences in the two types of tissue are at once apparent, even when allowances are made for the slightly smaller magnification in the case of mutton. The distortion in the mutton section is practically the same as that shown by the beef illustrated in figure 6, and having a very short latent period of freezing (0·75 hour). Mutton is thus much more resistant to slow freezing. The reason for this difference is at present unknown.

SUMMARY AND DISCUSSION.

In the experiments recounted in this paper it was found that:

(1) Increasing the latent period of freezing increased the changes in the beef, as determined by the quantity of drip, the nett nitrogen loss on thawing, and by the microscopic appearance in the frozen condition. In the frozen state, when the latent period of freezing was longer, the ice crystals were observed in smaller

numbers, but of larger size, whilst there were more bands of compressed "dehydrated" fibres, and fewer undistorted fibres than in the rapidly frozen samples. The disorganization of the muscle structure was also shown by the increase in the rate and in the extent of autolysis in the material after thawing.

(2) Marked differences were noted between the muscle tissue from animals of different age; in general, younger muscle could be frozen and thawed with less change than that from older beasts.

(3) The degree of reconstitution of the colloidal material was increased by increasing the latent period of thawing, since the quantity of drip was materially reduced when the frozen meat was thawed slowly. It could also be reduced by storage at 1°C. after thawing, showing that the imbibition of liquid by the colloid still went on after thawing was complete.

(4) In general the drip was less when the material was stored for a length of time not exceeding ten days at 1°C. before freezing, which may possibly be due to increased osmotic pressure in the muscle content.

(5) When frozen under similar conditions mutton showed much less ice formation and distortion of structure than beef.

The only experiments in which complete reversibility occurred, i.e., where there was no drip after thawing, and no apparent distortion of the frozen structure, were those in which liquid air was employed, giving an extremely rapid rate of freezing. The next best was obtained with a small piece by using a calcium chloride medium. This piece had a latent period of freezing of one-half hour. In this there was very little drip, and very little visible ice formation. To obtain such a rate with pieces of a size actually used in practice, such as quarters of beef, it would be necessary to use some freezing medium of approximately the same specific conductivity as saturated calcium chloride solution, and at a temperature considerably lower than —60°C. In addition to the mechanical difficulties involved, this would, according to Plank (10) introduce another factor in producing irreversibility, viz., that of low temperatures. Plank believes that some of the water in the muscle colloid is more closely bound to the colloid than the remainder, and that this bound water does not completely freeze out until a very low temperature is reached, viz., —56°C. When once frozen out, however, it is not readily reabsorbed during thawing. According to this view there is, therefore, a critical temperature below which this effect occurs. Some of the results quoted in Table 1 may possibly be attributed to this effect of low temperature. In experiments 14 and 23, for example, the same type of beef was employed, and it was frozen by means of a calcium chloride bath, in the former case at —10·5°C., and in the latter at —23° to —27°C. Both had approximately equal latent periods of freezing; yet the drip was considerably greater where the lower temperature was employed, even though there was a slower rate of thawing.

The conditions tested in this investigation which were found to affect the

quantity of drip, and the structural alteration, were therefore the rate of freezing, the rate of thawing, the time of storage after thawing, storage before freezing, and possibly the temperature to which the meat was reduced in the freezing process. Of these factors the first appears to be the most important.

The work, however, indicates that though many factors are involved, it may yet be possible to find a combination of conditions by which beef may pass through the refrigeration cycle with the minimum amount of change.

The authors desire to acknowledge the help afforded them by the A.N.R.C. Committee previously referred to, and also their appreciation of the very active interest taken by Mr. J. Hepburn, Works Manager and Chief Engineer, Victorian Government Cool Stores, who made the arrangements for the necessary refrigerating facilities. The investigation was facilitated by the generous co-operation of the Victorian Agricultural Department and the Institute of Science and Industry, and the University of Melbourne.

REFERENCES.

1. British Food Investigation Board, Special Report No. 7.
2. British Food Investigation Board, Annual Reports, 1922, 1923, 1924.
3. Callow, E. H.: Biochem. Journ., 19 (1925), p. 1.
4. Fearon, W. R. and Foster, D. L.: Biochem. Journ., 16 (1922), p. 564.
5. Kallert, E.: Zeitschrift für die Ges. Kälte-Industrie, 30 (1923), p. 77.
6. Kallert, E.: Zeitschrift für die Ges. Kälte-Industrie, 32 (1925), p. 8.
7. Love, E. F. J.: Proc. Roy. Soc. Vic., 38 (1925).
8. Moran, T.: Proc. Roy. Soc. B., 98 (1925), p. 436.
9. Plank, R.: Zeitschrift für die Ges. Kälte-Industrie, 32 (1925), p. 109.
10. Plank, R.: Zeitschrift für die Ges. Kälte-Industrie, 32 (1925), p. 141.
11. Plank, R. and Kallert, E.: "Uber die Behandlung und Verärbeitung von gefrorenem Rindefleisch," Berlin, 1916.
12. Richardson, W. D. and Scherubel, E. F.: Journ. Am. C.S., 30 (1908), p. 1515.

BLOOD-GROUPING OF AUSTRALIAN ABORIGINALS.

by

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(Submitted for publication 9th February, 1926.)

The Australian aboriginal is rapidly dying out. Comparatively few pure-blooded natives are now met with in the settled districts. In the central and northern parts of the continent their numbers are, however, still considerable.

The Australian native has apparently been almost completely, if not for practical purposes absolutely completely, isolated from other races of mankind for a period to be measured in tens of thousands of years. As remains of the dingo, his presumed companion when he arrived in Australia, are said to have been found in association with larva deposits, it is possible that he may have been in Australia when the extinct volcanoes in the Mount Gambier and Warrnambool district were in eruption. The other races of mankind have had frequent chances of mixing with each other, so that hybridization must have taken place to a greater or lesser extent.

From the results obtained in the blood-grouping of these various races there is a possible suggestion that they are composed of varying intermixtures of two sub-species of man. In the European at one end of the scale, one of these sub-species may exercise a more dominating influence, as shown in the blood-grouping, than is the case, say, in negroes or Hindus, towards the other end of the scale. This raises the question as to whether the Australian aboriginal may have been segregated at such an early stage that the intermixture of the two sub-species had not yet taken place, with the result that only one agglutinogen might be met with in an examination of individuals of this race. Under any circumstances, it was advisable to make a blood-grouping of the aboriginals, to place on record, before they disappeared, the relative proportions of the different groups met with in as large a number of individuals as possible.

During the last few years every opportunity has been taken, when making journeys in the back country, to ascertain the blood-groups of any pure-blooded natives met with. The result is that up to date 101 of such examinations have been made, and only two blood-groups have been met with—55 of the individuals belonging to Group II, and 46 to Group IV. It is the purpose of this short communication to place on record the results so far obtained.

When travelling in the interior it is often a difficult matter, especially in the open, to carry out successfully and in full detail all the tests that might be made. It is only found practicable, in the majority of cases, to take Type Sera II and III for testing purposes. After little, or sometimes much, persuasion, and the presentation of tobacco or sweets, and an explanation of the purpose in view presented in pidgin English, it has been possible to obtain the consent of individuals. A drop of blood was then drawn into a little citrate solution, and finally mixed with the respective sera on a glass slide by means of the end of a match. On rocking the slide clear-cut results were obtained.

Point McLeay Natives. With the assistance of Dr. Barlow, in 1922, 16 natives at Point McLeay, on Lake Alexandrina, were tested; 3 belonged to Group II and 13 to Group IV. Of the males, 3 belonged to Group II and 6 to Group IV. Of the females, 0 belonged to Group II, and 7 belonged to Group IV.

North-East of South Australia. On a visit in May, 1924, to the extreme North-east portion of South Australia, blood-groupings were obtained at Murnpeowie, Innamincka, Cordillo, and Arrabury, just over the Queensland border. Sixteen belonged to Group II and 12 to Group IV. Of the males, 5 belonged to Group II and 5 to Group IV. Of the females, 11 belonged to Group II and 7 to Group IV.

Tarcoola District. In November, 1925, Dr. Clark Wissler and Mr. E. Embree, representatives of the Rockefeller Foundation, were taken on a visit to Wilgena, near Tarcoola. Blood-groupings were made on this expedition of 55 of the natives, with the assistance of Professor Brailsford Robertson; 36 belonged to Group II and 19 to Group IV. Of the adult males 18 belonged to Group II and 8 to Group IV; and of the adult females 12 belonged to Group II and 8 to Group IV. Of the children of mixed sexes 6 belonged to Group II and 3 to Group IV. During the examination of these natives close observation was made of their physiognomy by Dr. Clark Wissler, Mr. Embree, and Professor Robertson, and these observers were under the impression that they could frequently foretell to what particular group an individual native would be found to belong. This, if correct, is exceedingly important, but will require much fuller investigation.

At the Adelaide Hospital, in 1923, one native, tested by Dr. Barlow, was found to belong to Group IV. Another, a member of the famous Arunta tribe, also belonged to Group IV.

The results may be shown in tabular form as follows:

	Group II.	Group IV. (Moss's Classification)	Total.
Point McLeay	3	13	16
N.E. of South Australia	16	12	28
Tarcoola District	36	19	55
Adelaide Hospital	0	2	2
	55	46	101

It will be seen that the relative proportions of these two groups vary somewhat in the different districts. In the southernmost (Point McLeay) there are only 3 of Group II and 13 of Group IV. In the N.E. of South Australia Group II slightly exceeds Group IV, and amongst the Tarcoola blacks Group II is nearly twice as numerous as Group IV. When the latter (the Tarcoola blacks) are divided further into males, females, and children, we find that the same relative proportion exists. The examinations made at Cordillo included a mother aged about forty and her twin daughters, aged seventeen; these all belonged to Group IV.

It thus appears that amongst the small number of natives (101) thus far examined by us in Southern Australia no member of Group I or Group III (Moss's Classification) has been met with. This part of Australia is considerably remote from the northern and north-eastern portions, where it is possible that recent admixtures with Malays and Papuans may have occurred.

Dr. A. H. Tebbutt (1, 2) has carried out a number of examinations on presumably full-blood Australian natives in New South Wales and Queensland. He has combined his results in the second of these two papers. He tested a total of 191, of which 14 were in New South Wales and 177 in Queensland. Transposing his groups from the Jansky to the Moss Classification, he found amongst the New South Wales natives 1 in Group I, 7 in Group II, 0 in Group III, and 6 in Group IV. Amongst the Queensland natives he found 1 in Group I, 66 in Group II, 11 in Group III, and 99 in Group IV. He thus met with 2 examples of Group I and 11 examples of Group III amongst 191 natives. It would thus seem as though all four groups are to be met with amongst pure-blooded Australian aborigines, unless the presence of Groups I and III is to be explained by a recent and slight intermixture with a foreign race coming from the north. In any case, our results show that the Australian native differs from all other races in having a biochemical index (iso-agglutinin index), as already pointed out by Dr. Tebbutt, higher than any other, and still higher than the English, which otherwise tops the scale. According to the blood-grouping the Australian native is ultra-European.

REFERENCES.

1. Tebbutt, A. H. and McConnel, Sarah V.: Med. Journ. of Aust., 1922, p. 201.
2. Tebbutt, A. H.: Med. Journ. of Aust., 1923, p. 346.

THE EFFECT OF THYROID FEEDING ON THE OESTROUS CYCLE OF THE GUINEA PIG AND MOUSE.

by

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(Submitted for publication 12th February, 1926.)

The relationship between the activity of the thyroid gland and the function of the ovaries in the human female is attested by a large amount of clinical evidence. The enlargement of the thyroid sometimes seen during menstruation and pregnancy, the occurrence of thyroid disorders at puberty and the climacteric, the delayed development of the genital organs in cretins, and the disturbances of menstrual function which frequently accompany thyroid disease, suggest such a relationship, though the profound disturbances of general metabolism which accompany alterations in the secretory activity of the thyroid may well account for all the observed changes in genital function.

The experimental results of thyroid feeding and of removal of the thyroid gland in animals are less clear. Hammett (9) showed that removal of the thyroid apparatus in rats 45 to 56 days old did not inhibit sexual activity or prevent reproduction, though breeding was delayed and fewer and smaller young were produced. In sexually mature albino rats, 100 to 150 days old, removal of the thyroid and parathyroid caused retrogression of the ovaries and uterus, while removal of the parathyroid alone had little effect. Alquier and Theuveny (4), using adult dogs, and Bensen (6), working with rabbits, found no alteration in ovarian structure after removal of the thyroid apparatus, though Hofmeister (12) had found evidence of follicular degeneration of the ovaries of rabbits after thyroidectomy. Evans and Long (14), using rats of various ages, found that thyroidectomy resulted in cessation of oestrus for from 6 to 27 days, after which the oestrus occurred normally. In young animals there was no effect on the time of maturity or on the length of the oestrus cycle, though in these, evidences of regeneration of thyroid tissue were found at autopsy, which may have explained the irregular results.

Herring (11) found that thyroid feeding in white rats caused an acceleration of the growth of the ovary, and Evans and Long (14) observed a prolongation of

the oestrous cycle or inhibition of oestrus when rats were fed with large doses of ox thyroid, though with small doses, sufficient to cause loss of weight and increased consumption of food, the oestrous cycles were not greatly disturbed.

In the present paper we have studied changes in the oestrous cycle of the guinea-pig and mouse, using a dosage of thyroid graded according to the weight of the experimental animal.

Methods.

The demonstration by Stockard and Papanicolaou (16) of a regularly recurring cycle of changes in the microscopical appearance of the vaginal secretion of the guinea-pig, correlated with structural alterations in the lining of the genital tract, has afforded a means of accurately determining the onset of oestrus and the duration of the oestrous cycle. The method has been found to be applicable in the pig (7), rat (14), mouse (1), opossum (10), rabbit (17), and in the cow (15). It is possible, also, by the vaginal smear method, to study the effect of various procedures on the function of the reproductive system, for it is reasonable to suppose that any marked disturbance will be reflected in the oestrous cycle.

Our work was done on virgin female white mice, three months old, of which ten were fed with thyroid extract, while five served as controls, the whole group of animals being kept under identical conditions and fed on bread and milk, sunflower seeds, and whole maize. Our guinea-pig experiments were carried out on eighteen animals, varying in weight between 420 and 708 grams, of which twelve were non-pregnant females, which were segregated with their offspring immediately after parturition, the young being removed when three weeks old, while six were virgin females. Eight of the non-pregnant but parous females were fed with thyroid, and the remaining four, together with the six virgin females, were used as controls. All the animals were fed on a diet of cabbage, carrot, and bran, and were kept under similar conditions.

The guinea-pig experiments extended over a period of 64 to 113 days, while the mice were observed for 240 days.

Vaginal smears were made daily throughout the experiments. These were easily obtained by the use of a small piece of cotton wool tightly wound round fine forceps, and inserted high up into the vaginal canal. Smears were stained for two minutes with undiluted Leishman's stain and subsequently washed with tap water. This stain gives a more brilliant picture than the methylene blue used by earlier workers. Leucocytes show a deep purple nucleus, cornified vaginal cells are unstained, whilst recently desquamated vaginal and uterine cells show faint pink cytoplasm with blue nuclei. Figures 1 to 4 illustrate very well the changes occurring in the vaginal secretion during the oestrous cycle of the mouse.

Throughout the period of thyroid feeding we have employed carefully graded

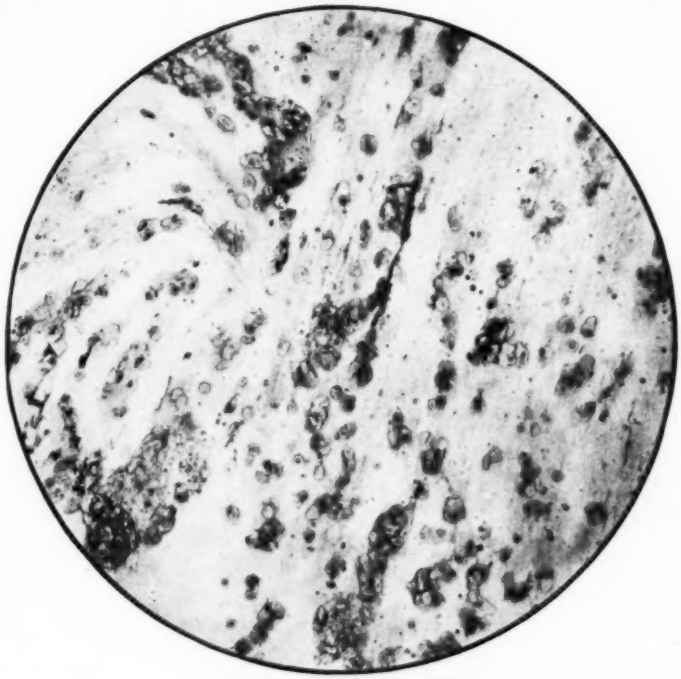

Fig. 1. Magnification 72.

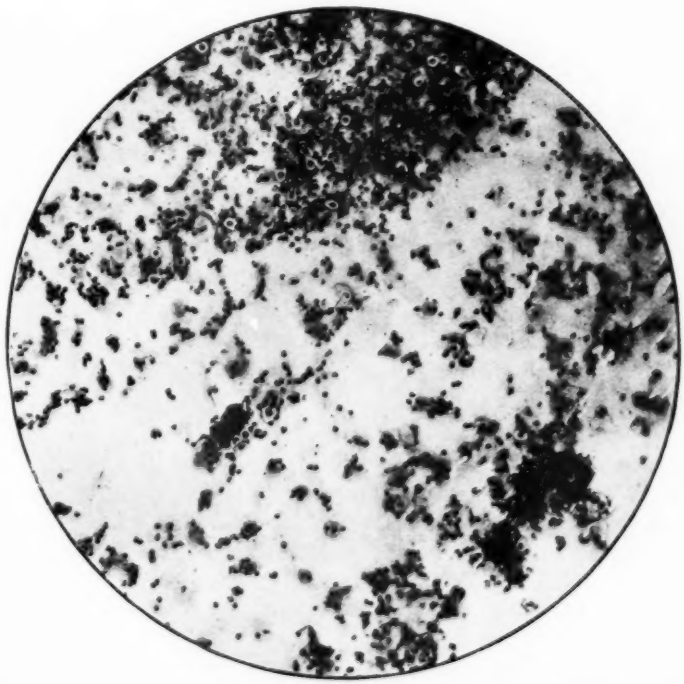

Fig. 2. Magnification 72.

Micro-photographs of typical smears at the various stages of the cycle in the mouse.

doses of thyroid extract. Burroughs, Welcome's preparation, containing not less than 0·05 per cent. of iodine, was used. A tablet containing 0·002 grams of dried thyroid was specially prepared for us by this firm, so that we were able to employ a dosage of one forty-thousandth to one ten-thousandth of the body weight. We usually commenced with the smaller dose, and gradually increased it. No difficulty was experienced in feeding the guinea-pigs, but with mice we had

TABLE I

Weight in grams	Number of days observed	Duration of cycles in days	Average cycle in days	Duration of oestrus in days	Average oestrus in days
		CONTROLS			
545	64	16 16 16	16	2 2 1	1·7
470	64	16 17 16	16·3	1 2 2	1·7
532	64	16 17 17 18	17	1 2 2 1	1·5
420	64	16 17	16·5	2 2	2
GROUP AVERAGE			16·5		1·73
320	113	17 16 15 15 16 16	15·8	1 1 2 1 2	1·3
355	113	18 16 15 16 15 17 16	16·1	1 1 2 2 2 1 2	1·5
340	113	17 16 15 15 15 15	15·5	1 2 2 1 2 2	1·7
295	113	17 15 16 15 15 15 16	15·5	1 1 1 1 2 2 2	1·4
425	113	17 16 15 15 16	15·8	1 2 2 2 2	1·8
365	113	16 17 15 15 15 15	15·5	2 1 2 2 2 2	1·8
GROUP AVERAGE			15·7		1·6
		THYROID FED			
500	64	16 16 17	16·3	3 4 5	4
547	64	15 16 17	16	4 4 5	4·3
640	64	15 15 16 17	15·75	4 5 5 5	4·8
422	64	16 17	16·5	4 5	4·5
708	64	16 17 17	16·6	5 5 6	5·3
477	64	15 16	15·5	5 5	5
620	64	15 16 16 16	15·75	4 4 5 5	4·5
485	64	16 17 16	16·3	4 5 6	5
GROUP AVERAGE			16·1		4·7

some trouble until we devised the plan of grinding the thyroid extract up with a small quantity of condensed milk, which the animals took readily. The potency of this preparation was shown by the occurrence of the classical signs of experimental hyperthyroidism in both species of animals—loss of weight, falling out of hair, increase of heart rate, transient attacks of diarrhoea, with loss of strength and diminished activity. Occasionally exophthalmos was noted in the guinea-pigs.

Results.

Our observations on guinea-pigs are set out in Table 1. Of the controls, the first four are non-pregnant parous females, and the remainder virgin guinea-pigs. It will be noted that the length of the oestrous cycle in the last group averaged 15·7 days, as compared with 16·5 days, the average in the other four controls. The average duration of oestrus was also short in the virgin guinea-pigs, 1·6 against 1·73 days. These results are in complete agreement with those of Stockard and Papanicolaou (16), who first used the method in the normal guinea-pig.

In the thyroid-fed animals the average length of the oestrous cycle was 16·1 days, and showed no significant change from the corresponding normals, but there was a definite increase in the duration of the oestrus to an average of 4·7 days. Our observations are somewhat incomplete in regard to the length of time during which the animals were under observation, and our experiments with the mouse, which is a more suitable animal for such experiments. having an oestrous cycle of four to six, instead of fifteen to seventeen days in the guinea-pig, were carried out for a much longer period.

In these later experiments we observed our thyroid-fed animals both before and after thyroid feeding, as well as control animals examined daily over the whole period to exclude any possible seasonal variation during the time of the experiment (240 days).

The results are graphically recorded in figure 5. Since the smears were made at the same time on each successive day, the records give a fairly accurate picture of the occurrence of oestrus. The animals were divided into three groups, each containing five animals. Each group was kept in a separate pot. A and B were the experimental animals and C the controls.

The control animals during the whole period had an average length of cycle of 6·7 days, and an average duration of oestrus of 2·4 days. During the first 72 days, in which no thyroid was administered to the experimental animals, the figures for the controls were 6·3 and 2·2 days. During the second period of 115 days the average figures were 6·8 and 2·8 days, and during the last period of 53 days the averages were 6·9 and 2·3 days. Apart from individual variations these figures do not suggest any marked seasonal change, though the increasing age of the animals may account for a small prolongation of oestrus and lengthening of the whole cycle.

The thyroid-fed animals during the first period when no thyroid was administered, had an average length of oestrous cycle of 6·5 days and an average duration of oestrus of 2·2 days, which did not differ significantly from the averages of the controls, 6·3 and 2·2 days. During the second period of 115 days there was a definite increase in the cycle to an average of 12·9 days, while the oestrus itself was increased to 4·2 days. The change was sudden in its onset, occurring within

Fig. 3. Magnification 59.

Fig. 4. Magnification 42.

Micro-photographs of typical smears at the various stages of the cycle in the mouse.

the first few days after thyroid feeding had commenced. Individuals showing irregularities in rhythm showed them more markedly when under the influence of thyroid. The first change obvious during the first 40 days was an increase in the length of the oestrus without any striking increase in the dioestrus. After this time the lengthening of the whole cycle was due almost entirely to lengthening of the dioestrus, and the oestrus tended on the whole to be shortened or at all

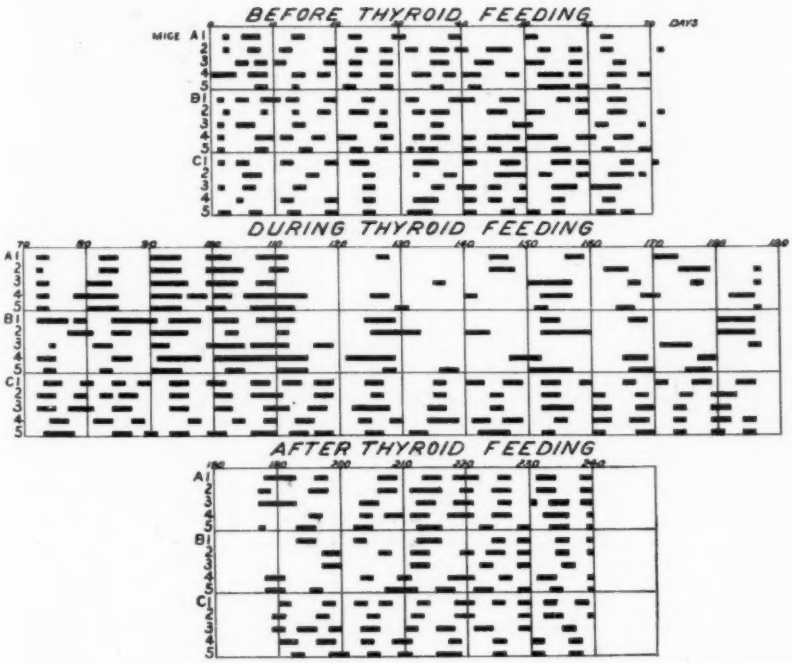

Fig. 5.

events not notably increased. A comparison of the average figures for the first 40 days with those for the remainder of the period shows this in striking fashion, the length of the oestrous cycle and the duration of oestrus during the former being 9·4 and 4·3 days respectively, and 16·4 and 4·0 days respectively for the latter.

With the cessation of thyroid feeding there was a rapid return to normal rhythm. For the whole period of 53 days the average length of the cycle was 7·8 and the duration of oestrus 2·9 days, as compared with corresponding figures for the controls, 6·9 and 2·3 days. The return to normal rhythm was complete

23 days after feeding with thyroid was discontinued; the average figures for the last 30 days being 6·7 and 2·4. The average figures for the last 40 days were 7·2 and 2·7 days, so that after 13 days the reversion to normal rhythm was almost complete.

It is not possible as yet to advance a definite explanation of these undoubted effects of thyroid feeding, since we know nothing with certainty of the manner in which the cycle is normally controlled. Many observers, following Beard (5) and Fraenkel (8), consider that the corpus luteum is of primary importance in the determination of the characteristic periodic changes in the genital tract. Leo Loeb (13) has brought forward experimental evidence in favour of such a view, though his results have been severely criticized by Stockard and Papanicolaou (16). Most writers, however, admit that a hormone elaborated by the corpus luteum appears necessary for the normal development of the uterine and vaginal mucosae, and that the absence of this secretion leads to regression and degeneration of the uterine tissue. The Graafian follicles have also to be considered in this connection, for, though the corpus luteum undergoes degeneration at the height of oestrus, the follicles at this stage attain their greatest development. Allen, Doisy, and their collaborators (2, 3) claim that they have isolated from this source, though not from the corpus luteum, a hormone which on injection into spayed mice and rats brings about the usual phenomena of oestrus.

It remains an open question whether thyroid extract has a specific effect on the reproductive organs, either directly in the production of ovarian hormones, or indirectly by sensitizing the uterine and vaginal mucus membrane to them; or whether it exerts its effect by its influence on general metabolism or on the activity of other ductless glands which play a subsidiary part in the mechanism of reproduction.

CONCLUSIONS.

1. The administration of thyroid extract causes in mice an increase in the duration of oestrus and in the length of the whole cycle.

2. In the guinea-pig there is a marked change in the length of oestrus but no striking increase in the duration of the cycle.

ACKNOWLEDGMENTS.

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REFERENCES.

1. Allen, E. A.: Amer. Journ. Anat., 30 (1922), p. 297.
2. Allen, E. A., Pratt, J. and Doisy, E. A.: Journ. Amer. Med. Ass., 85 (1925), p. 399.
3. Allen, E. A., Doisy, E. A., *et alia*: Amer. Journ. Anat., 34 (1924), p. 133.
4. Alquier, L. and Theuveny, L.: Comp. Rend. Soc. de Biol., 66 (1901), p. 217.
5. Beard, J.: Anat. Anz., 14 (1898).
6. Bensen, W.: Virchow's Archiv., 172 (1902), p. 229.
7. Corner, G. W.: Publication 276, Carnegie Institute of Washington (1921), p. 117; Corner, G. W. and Amsbaugh, A. E.: Anat. Rec., 12 (1917), p. 287.
9. Hammett, F. S.: Journ. Metabol. Res., 3 (1923), p. 417; Amer. Journ. Anat., 32 (1923-24), p. 37.
10. Hartman, C.: Amer. Journ. Anat., 32 (1923-24), p. 353.
11. Herring, P. T.: Quart. Journ. Exp. Physiol., 11 (1917), p. 231.
12. Hofmeister, F.: Beitr. z. Clin. Chir., 11 (1894), p. 441.
13. Loeb, L.: Deutsche Mediz. Wochnschr., 1 (1911); Journ. Morphol., 22 (1911).
14. Long, G. A. and Evans, H. M.: Memoirs of the University of California, 6 (1922), p. 1.
15. Murphey, H. S., McNutt, G. W., Zupp, B. A. and Aitken, W. A.: Anat. Rec. 29 (1925), p. 370.
16. Stockard, C. R. and Papanicolaou, G. N.: Amer. Journ. Anat., 22 (1917), p. 225.
17. Wood, G. J.: Anat. Rec., 29 (1925), p. 309.

LEGENDS.

Micro-photographs of typical smears at the various stages of the cycle in the mouse.

1. At the height of oestrus.

2. At the fourth stage of oestrus, when the leucocytes predominate and the uterine cells are disappearing.

3. In dioestrus, shortly after oestrus.

4. At a somewhat later period in dioestrus.

Figure 5. Graphical representation of observations on the duration of oestrus in the mouse.